comprehensive analysis of the issue can afford to exclude it. Much Renaissance art consists of depicting the same pose repeatedly until a canonical form is achieved, as in the *Madonna and Child* or *Christ on the Cross*.

In addition to the peak shift principle, the authors outline seven other principles (drawn from Gestalt psychology and neurology) that they believe constitute the deep structure of the art experience. For example, their second (grouping) principle proposes that multiple features are grouped together by the visual system into unified objects and that this grouping is reinforced by connections between the brain's visual system and limbic system (which controls emotion). Taking examples from the entire history of art, the case for each principle is developed with ingenuity and flair. These two neurologists reveal much more of the brain processes that are likely to be involved in implementing these principles than would most art critics. But it is unlikely that many art critics would regard these eight laws as exhausting the repertoire of principles of art, even accepting the caveat that there is much that is individual in art and that is not amenable to a principled analysis. Notably, Ramachandran and Hirstein say nothing about the widely recognized principle of balance in composition, of the power of the center emphasized by the American art critic R. Arnheim (*5*), or of the dynamic interplay of visual forces emphasized by the father of modern abstract art, W. Kandinsky (*6*).

Of the eight principles delineated, the one that may be of most interest to artists is that of perceptual problem solving (where the subject matter of the artwork can be extracted only with some effort rather than being immediately obvious). Curiously, the section addressing this principle seems to have been lost in the main text, but it is clearly identified in the summary. Had the authors included a section on perceptual problem solving, perhaps it would have explored how this principle accounts for many of the diverse manifestations of 20th-century art. One of the characteristics of modern art is that it encompasses (among other things) a succession of styles of abstraction: fauvism, cubism, dadaism, futurism, abstract impressionism, op art, minimalism, and so on. Even before the 20th century, there existed extremes of representational style such as impressionism, pointillism, expressionism, photo-realism, and so on, that are themselves forms of abstraction. The point is that any degree of abstraction requires the viewer to make a perceptual effort to extract the theme of the painting (compared with the essentially effortless perception of representational art). This effort itself forms an essential component of the artistic experience—by slowing down the perceptual processes of decoding the artwork the viewer becomes aware of their evolution and interplay over time, and then experiences a sense of achievement when the full composition falls into place (or of continued mystery if it does not). Thus, the problem-solving principle, evoked to account for the appeal of hiding the female form under diaphanous garb, could account for much of the development of 20th-century abstract movements (which are difficult to interpret in terms of the other seven principles).

Ramachandran and Hirstein state that their goal is to foster debate on the principles underlying the artistic experience. This they undoubtedly do, as exemplified by the Commentaries and Letters that are to be found in the same issue. But it is clear that the debate will extend far beyond the eight principles that they have enunciated.

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PERSPECTIVES: BIOMEDICINE

Lentiviral Vectors—the Promise of Gene Therapy Within Reach?

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The modification of the genetic material of living cells for therapeutic purposes still remains an unrealized promise as a medical intervention in humans. In December 1995, researchers in the gene therapy community received a wake-up call when the National Institutes of Health issued a report that criticized the premature implementation of gene therapy clinical studies and called for a return to the drawing board to optimize gene delivery (vector) systems. Up to that time, scientists had relied mainly on murine retroviruses such as Moloney murine leukemia virus for applications that required stable gene transfer (transduction) into the chromosomes of target cells, and on the high efficiency of adenovirus vectors when genomic integration was not a requirement. However, the use of murine retroviral vectors in human protocols has been associated with poor efficiency of gene transfer, probably because these vectors only integrate into the genome of cells that are actively dividing (*1*). This limitation has prompted a search for efficient vectors that are capable of delivering and expressing genes in nondividing cells.

Considerable progress has been made on this front with the optimization of two important vector systems based on adeno-associated virus and lentiviruses, such as human immunodeficiency virus (HIV). Adeno-associated vectors have been used successfully to treat hemophilia B (a bleeding disorder caused by a deficiency in coagulation factor IX) in dogs by portal vein delivery of the vector to the liver and by its direct injection into muscle (*2*). Plans are under way to test an adeno-associated viral vector carrying the human factor IX gene in hemophiliac patients. Lentiviruses are a type of retrovirus that can infect both dividing and nondividing cells. They have proven extremely efficient at providing long-term gene expression (for up to 6 months) in a variety of nondividing cells (such as, neurons and macrophages) in animal models. Progress is under way to ensure the safe and efficient production of lentiviral vectors for future human use.

Unlike murine retroviruses, HIV and other lentiviruses have a complex genome that, in addition to the essential structural genes (*env*, *gag*, and *pol*), contains regulatory (*tat* and *rev*) and accessory genes (*vpr*, *vif*, *vpu*, and *nef*). HIV has evolved to efficiently infect and express its genes in human cells, and is able to infect nondividing cells such as macrophages because its preintegration complex can traverse the intact membrane of the nucleus in the target cell (see the figure). This complex is composed of the enzyme integrase, the product of the *vpr* gene, and a protein encoded by the *gag* gene called matrix. The matrix protein contains a localization sequence that is recognized by

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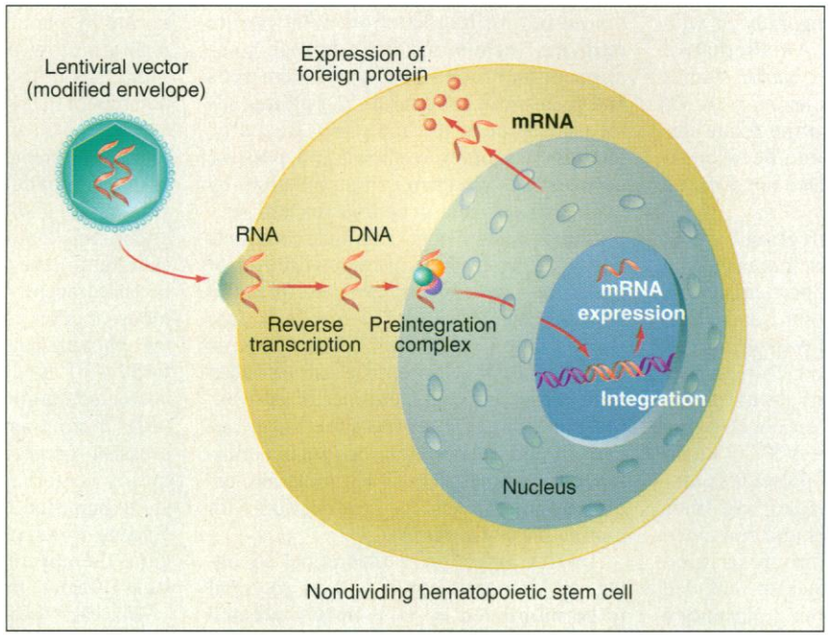
the nuclear import machinery, which docks the complex at a nuclear membrane pore enabling the preintegration complex to pass into the nucleus (*3*). Lentiviruses cannot efficiently transduce truly quiescent cells (cells in the G_0 state) owing to a block at the reverse transcription step (when the RNA viral genome is transcribed into DNA) (*4*), and they require progression through at least the G_{1b} stage of the cell cycle (during which active RNA synthesis takes place) (*5*). However, unlike murine retroviral vectors, HIV-based vectors can achieve effective and sustained transduction and expression of therapeutic genes in nondividing cells, such as hematopoietic stem cells (*6*, *7*) and in terminally differentiated cells such as neurons (*8*), retinal photoreceptors (*9*), muscle, and liver cells (*10*).

Although lentiviral vectors hold promise as delivery vehicles for gene therapy, their parental origin has raised considerable safety concerns that have so far precluded human testing. Chief among these is the possibility that a lentivirus that is able to self-replicate could be produced during manufacture of the vector in the packaging cell line or in the target cells by a process of recombination. A self-replicating infectious vector could conceivably cause cancer by inserting itself into the host genome in a way that results in the activation of a neighboring proto-oncogene (insertional mutagenesis), and could potentially transfer its genetic material to germ line cells. In addition, such infectious lentiviral vectors could potentially behave in humans like HIV. Having provided proof of principle that these vectors are efficient gene delivery vehicles, the field has turned its attention to the development of newer vectors and production systems with built-in safety features to prevent the emergence of replication competent lentivirus (RCL).

In most laboratory applications, lentiviral vectors are generally created in a transient transfection system in which a cell line is transfected with three separate plasmids. The vector plasmid contains genetic cis-acting sequences necessary for the vector to infect the target cell and for transfer of the therapeutic (or reporter) gene. The packaging plasmid contains the elements necessary for vector packaging such as structural proteins (except for env) and the enzymes required to generate vector particles (*11*). The lentivirus *env* gene is deleted from the packaging plasmid (otherwise the vector would only be able to infect $CD4^+$ T cells) and instead the envelope gene of a different virus is supplied in a third plasmid. A commonly used envelope gene is that encoding the G glycoprotein of the vesicular stomatitis virus (VSV-G), which confers stability to the particle and permits the vector to be concentrated to high titers (*11*, *12*). The use of three separate plasmids and the absence of overlapping sequences between them minimizes the possibility of recombination during vector production. In addition, because no viral proteins are expressed by the lentiviral vector itself, they do not trigger an immune response against cells expressing vector in animal models (a particular problem with vectors based on adenovirus).

The initial packaging plasmids contain most HIV genes except for *env*. In an effort to improve safety, subsequent HIV vectors have been produced in which the packaging plasmid is devoid of all accessory genes. This process does not interfere with efficient vector production and significantly increases the safety of the system because potential RCLs lack the accessory genes necessary for efficient replication of HIV in humans. Although these vectors can transduce growth-arrested cell lines and neurons in vivo, they do not efficiently transduce macrophages, a result consistent with the known need of the accessory gene *vpr* for HIV infection of these cells (*13*). The requirement of *vpr* or *vif* for efficient transduction of liver cells has also been reported (*10*). These results indicate that the requirement of accessory genes for efficient lentivirus-mediated gene transfer is dependent on the type of cell chosen as target, suggesting that future applications of lentiviral vectors will depend on vector constructs with different accessory genes.

Lentiviral vectors engineered to become packaged into virions in the absence of the regulatory gene *tat* have also been described. In these vectors the *tat* gene has been removed from the packaging plasmid, eliminating an essential virulence factor that could contribute to a possible RCL (*14*, *15*). The latest development in the quest for a safe system is the so-called self-inactivating vector (*15*). This vector contains a deletion in a region at the end of the virus genome called the long-terminal repeat (LTR). LTRs are unique cis-acting sequences that flank the virus genome and are essential to the virus life cycle. A sequence within the upstream LTR serves as a promoter under which the viral genome is expressed. The deletion introduced in the downstream LTR is transferred to the upstream LTR during reverse transcription. This deletion inactivates the LTR promoter and eliminates the production of vector RNA. The gene to be transferred (a reporter or therapeutic gene) is expressed from an exogenous viral or cellular pro-



Lentiviral voyager. Gene transfer by a lentiviral vector. Vectors based on lentiviruses such as HIV are able to infect both dividing and nondividing cells. After the lentiviral vector has infected, for example, a nondividing (resting) hematopoietic stem cell, the vector RNA containing the therapeutic gene is transcribed into DNA. The DNA forms a preintegration complex with the accessory protein Vpr, the enzyme integrase, and the protein matrix. The localization sequences of these proteins enable the preintegration complex to cross the nuclear membrane (vectors based on other viruses must wait for the nuclear membrane to break down during cell division before they are able to access the host DNA). Once inside the nucleus, the DNA is inserted into the host genome by integrase.

moter that is inserted into the lentivirus vector. As lentiviruses integrate randomly into the host genome, an important safety feature of these vectors is that inactivation of the promoter activity of the LTR reduces the possibility of insertional mutagenesis. In addition, because the expression of the vector RNA is eliminated, the potential for RCL production in the target cell is further minimized. Other modifications to enhance safety and specificity include the use of specific internal promoters that regulate gene expression, either temporally or with tissue or cell specificity. An alternative strategy to improve safety in human studies would be to use nonhuman lentiviruses. Of these, vectors derived from the feline immunodeficiency virus have been engineered to efficiently transduce nondividing human cells (*16*).

As the transient transfection system used for virus production increases the chances of recombination, particularly after the system is scaled up for human use, researchers have designed packaging cell lines that continuously produce high-titer vector. The development of efficient packaging lines has proven challenging because expression of the VSV-G envelope and a number of HIV proteins is toxic to cells. The initial packaging lines produced virus at low titers and contained the HIV envelope, which restricted tropism of the vector. They also included the *env* gene in the packaging plasmid, a feature that increased the potential for RCL to arise. Recently, a producer line has been designed in which the expression of packaging genes and VSV-G, and therefore the production of vector, can be turned on at will (*17*). The cell line can be expanded for scale-up vector production when the expression of toxic genes is turned off. This cell line produces high titer vector without generating RCL. The development of efficient and safe packaging systems will facilitate large-scale production of vector that can be tested for safety before its use in human trials. Although the potential for toxicity resulting from the development of RCL has been minimized with novel vectors and producer cells, the ultimate safety test will require the use of these vectors in the clinic. It is noteworthy that even the early lentiviral systems have not generated RCL in vitro or in vivo. We have transplanted hematopoietic stem cells transduced with an HIV vector into rhesus macaques with a 14-month follow-up to date. This procedure proved to be safe; all animals in this study have remained healthy without evidence of circulating HIV or vector (*18*).

Many gene therapy protocols have been designed to correct a number of inherited metabolic, infectious, or malignant diseases using the coveted target for gene modification, the hematopoietic stem cell. This cell has the capacity to self-renew and to differentiate into all of the mature cells of the blood and immune systems. Many diseases that affect these systems could potentially be treated by the stable introduction of therapeutic genes into stem cells. However, the need to activate stem cells with growth factors to induce cell division before transduction with murine retroviral vectors, results in lineage commitment and differentiation of stem cells and their weak long-term engraftment after transplant to the recipient. Recently, lentiviral vectors were shown to bypass the need for ex vivo stem cell stimulation, by mediating efficient gene transfer into very primitive human stem cells that contributed to stable, long-term reconstitution of SCID mouse bone marrow with many hematopoietic lineages (*6*). Similarly, in a rhesus macaque model of autologous transplantation with lentivirus-transduced stem cells, we found multilineage gene expression, suggesting transduction of an early blood cell progenitor under conditions of minimal stem cell stimulation, ordinarily insufficient for transduction with murine retroviruses (*18*).

In HIV infection, a theoretical advantage of lentiviral vectors is their potential to be mobilized by HIV in the infected patient, because the virus supplies all of the necessary elements for packaging of the vector. If these mobilized vectors contained the HIV envelope, they could efficiently transfer their genes (for example, genes custom-designed to confer resistance against HIV) into $CD4^+$ T cells, protecting them from subsequent HIV infection. Lentiviral vectors can also be designed to efficiently express their genes only in $CD4^+$ T cells that are infected with HIV (so called *tat*-inducible vectors). In these vectors, all HIV genes, including *tat* and *rev*, are ablated; cis-acting sequences required for integration, expression, and packaging are retained, and expression is dependent on the activity of the HIV LTR (which requires transactivation by Tat). We have shown that in this system, vector expression is induced efficiently upon HIV infection. Moreover, in the absence of genes that confer resistance against HIV, stable integration of this vector in permissive cell lines resulted in inhibition of HIV replication (*19*). Although the mechanism of HIV inhibition has not been completely elucidated, preliminary results suggest that this vector competes with HIV at the level of reverse transcription.

A number of other potential medical applications, where the modification of the genetic material of quiescent cells could result in the prevention or reversal of a disease process, are beginning to be explored. For example, the finding that lentiviral vectors can mediate stable and long-term gene transfer by direct injection of vector into the rat and mouse retina has lent support to the notion of gene therapy for the treatment of retinitis pigmentosa. This degenerative disease of the retina is characterized by photoreceptor cell death, resulting in a slow progression to blindness. Mutations in the cGMP phosphodiesterase β subunit (PDEβ) gene of rod photoreceptors lead to an autosomal recessive form of retinitis pigmentosa in humans, and in the *rd* mouse model of the disease. Previous studies have shown that adenovirus and adeno-associated virus–mediated PDEβ subretinal gene transfer results in a delay in photoreceptor cell death. Using the *rd* mouse model, a recent study demonstrated that photoreceptors could be rescued in up to 50% of eyes injected with a lentivirus vector containing the murine PDEβ gene (*20*). In contrast with the short-term expression previously obtained with adenovirus vectors, PDEβ expression in this study persisted for at least 24 weeks. This finding hints at the potential success of gene therapy in a disease that currently lacks effective treatment.

For each theoretical concern raised over the safety of lentiviral vectors, there are equally persuasive arguments that the concern can be addressed through vector design and experimental investigation. Ultimately, if their safety can be established in human trials, lentiviral vectors, with their ability to efficiently transfer genes long-term by in situ delivery, are well poised to help transform the promise of gene therapy into reality.

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